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Variable Patterns of Immunoglobulin and Complement Deposition in the Kidneys of Patients with Systemic Lupus Erythematosus

D. Koffler, MD, V. Agnello, MD, R. I. Carr, MD, and H. G. Kunkel, MD

GLOMERULAR LOCALIZATION of γ-globulin and complement has been observed in the late stages of the glomerulonephritis associated with systemic lupus erythematosus (SLE).[1-4] There is considerable evidence to suggest that these deposits result from the deposition of circulating antigen-antibody complexes along the glomerular basement membrane (GBM).[4-6] Several questions related to the renal localization of immune complexes during the early stages of the disease have not been answered. First, are immune complexes formed in patients without renal disease and deposited in the kidney? Second, is there a detectable point at which the glomerular localization of immune complexes induces functionally significant renal disease?

The present study indicates that there is a progressive renal deposition of immunoglobulins and complement which may be correlated closely with the clinical course of the disease. The mesangium appears to be the initial site of localization of protein followed by granular deposits along the basement membrane, and finally, in the advanced forms of nephritis, a coalescence of these deposits occurs with the formation of coarse, lumpy aggregates of protein.

From The Rockefeller University and the Department of Pathology, The Mount Sinai School of Medicine of the City University of New York, NY.

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Address for reprint requests: Dr. D. Koffler, Department of Pathology, The Mount Sinai School of Medicine, 5th Ave and 100th St, New York, NY 10029.

Materials and Methods

Renal and skin biopsies

Percutaneous renal biopsies were obtained from 19 patients and skin biopsies from 2 patients with clinical and serological evidence of SLE (Table 1). No evidence of significant renal disease was observed in 8 patients when the following parameters were used: (1) urinary 24-hr protein of less than 500 mg; (2) normal blood urea nitrogen; (3) absence of glomerulitis on microscopic examination of hematoxylin- and eosin-stained paraffin or cryostat sections of kidneys.

Proteinuria was manifest in 11 patients. Renal disease developed coincidentally with systemic symptoms in 2 patients, within 1–2 years in 3 patients, and more than 3 years after the onset of SLE in 6 patients. Only 1 patient showed signs of renal insufficiency manifested by an elevated blood urea nitrogen. Microscopic examination of the biopsies obtained from patients with proteinuria revealed focal glomerulitis, glomerular adhesions, basement membrane thickening, and in one instance, fibrinoid necrosis.

Necropsy tissues

Renal tissues were obtained at necropsy from 2 patients with SLE. The first was a 61-year-old female with positive LE tests for 5 years associated with arthritis, who died of congestive heart failure without evidence of renal disease. The second was a 24-year-old female with positive LE tests for 5 years associated with arthral-

Table 1. Immunofluorescence and Clinical Data on Renal Biopsies

Patient	Deposition pattern of γG-Globulin	24-hr urinary protein (g)	BUN (mg/100 ml)	Protein deposits* γG	γM	B_1C	Duration of disease (years) SLE	Renal disease
G.M.	Membranous	.10	18	2+	0	0	10	—
D.S.	Membranous	.20	13	3+	0	0	3	—
C.B.	Membranous	.43	18	1+	0	0	1	—
S.V.	Mesangial	.10	19	3+	0	3+	4	—
A.E.	Mesangial	.10	25	2+	±	2+	2	—
D.B.	Mesangial	.31	15	3+	1+	3+	6	—
P.W.	Mesangial	.33	26	2+	±	2+	1	—
L.D.	Mesangial	.44	20	2+	2+	2+	10	—
A.F.	Granular	1.20	3	2+	0	1+	4†	2
J.B.	Granular	1.50	14	2+	±	2+	2	1
H.M.	Granular	2.10	11	3+	3+	2+	7	2
J.B.	Granular	2.20	18	3+	0	2+	7	4
H.W.	Granular	2.60	12	3+	1+	3+	4	4
M.F.	Granular	4.00	12	3+	0	3+	7	2
M.M.	Granular	5.00	20	2+	2+	3+	5	1
E.B.	Granular	11.00	25	3+	1+	3+	10	2
M.M.	Granular	3+‡	13	2+	1+	2+	5	4
D.M.	Granular	3+‡	18	3+	2+	3+	3	3
M.R.	Lumpy	7.60	32	1+	3+	3+	12	2

* Graded as 0 indicating negative; ±, trace; 1+, minimal; 2+, moderate; and 3+, marked.
† Discoid LE for 4 years.
‡ Sulfosalicylic acid determination (24-hr urinary protein not available).

gias, pleuritis, pericarditis, leukopenia, and anemia. No evidence of renal disease was observed, but she developed a staphylococcal septicemia while being treated with steroids and expired.

Immunofluorescence studies

Renal biopsies were embedded and rapidly frozen in dry ice and isopentane at −70°. Fluorescein-labelled anti-sera to γG, γM, γA and β_1 C-globulins were prepared and utilized as previously described [4] at a fluorescein to protein ratio of 2:3. Aggregated γ-globulin was prepared by heating Fraction II for 10 min at 63° and then labelling with fluorescein at a concentration of 10 mg/ml of protein. A Leitz Labolux fluorescence microscope with 2 mm BG-12 exciter filter and a 490 or 510 mμ barrier filter was used. Ansochrome 200 film was used for photography.

Elution studies

Elution studies were performed on one biopsy specimen and two kidneys obtained at necropsy. The biopsy (Patient D.S.) was serially sectioned and 20 sections were treated with 10 cc of 0.02 M, pH 3.2 citrate buffer. The eluate was dialyzed against pH 7.2 phosphate buffered saline and was concentrated to 1/20 volume. Glomeruli from necropsy kidneys were isolated and eluted with acid buffer as previously described.[6]

Results

Immunoglobulins and complement localization

Distribution within glomeruli. Four distinct patterns of glomerular staining for γG-globulin were observed (Table I). Membranous localization was found in three renal biopsies, mesangial deposits in four biopsies, granular deposits in ten biopsies, and lumpy deposits in one biopsy. Mesangial deposits were characterized by irregular strands of protein lying between capillary loops, which appeared to radiate from the central portion of the glomerulus (Fig 1). Linear deposits appeared as a fine homogeneous staining of GBM (Fig 2). Granular deposits had a punctate pattern of fluorescence comprised of granules which varied in size and were distributed irregularly throughout the glomerulus (Fig 3). In one biopsy from a patient with nephrotic syndrome, a regular distribution of small granules was noted along the GBM (Fig 4). A biopsy from a patient with renal failure revealed coarse, lumpy deposits of γ-globulin which outlined the glomerular tufts and obscured the normal architecture of the glomeruli (Fig 5). These were similar to those observed in nephritic kidneys obtained at necropsy.[4]

Gamma-M-globulin and β_1C-globulin were observed in a similar distribution as γG-globulin, except for the linear staining pattern which was confined to γG-globulin. Mesangial deposits of immunoglobulins and complement were usually present, in addition to the linear, granular, or lumpy type of staining.

Two kidneys obtained at necropsy also showed linear deposits of γG-globulin along the GBM. One patient exhibited γ-globulin localization in the renal tubular basement membrane and Bowman's capsule in addition to GBM staining.

The pattern of distribution for immunoglobulins was correlated with the presence of renal disease (Table I). Linear and solitary mesangial deposits were found in kidneys without clinical or histological evidence of glomerular injury. These patients had 24-hr urinary protein levels of less than 500 mg. Granular deposits of immunoglobulins and complement were associated with the appearance of glomerulonephritis. In this group of patients 24-hr urinary protein values ranged from 1.2 to 11.0 g; BUN was within normal limits. One patient with lumpy deposits had marked proteinuria and a slight elevation of BUN.

There was no correlation between the length of systemic disease and the type of glomerular deposition of protein observed. Two patients had SLE for 10 years without clinical evidence of renal disease and showed a linear or mesangial pattern of staining for γG-globulin. In 3 patients with evidence of renal disease for a period of 4 years, distinct granular deposits without coalescence were evident. The most severe glomerulonephritis was observed in a patient with systemic symptoms for 10 years and a 2-year history of renal disease.

Skin biopsy results. Skin biopsies obtained from 2 patients (G.M. and D.S.) with linear deposits of γ-globulin and complement in renal glomeruli showed deposition of γ-globulin but not complement along the cutaneous basement membrane of segments of skin without histological evidence of inflammatory change.

Type of immunoglobulin present. Gamma-G-globulin was the major immunoglobulin deposited in 15 of the 19 renal biopsies studied (Table 1). In three biopsies approximately equivalent staining for γG-globulin and γM-globulin was noted, and in one case γM-globulin was the predominent immunoglobulin localized. Trace amounts of γA-globulin were found in mesangial deposits. Glomeruli containing significant amounts of γM-globulin were incubated with fluorescein-labelled aggregated γ-globulin. In 1 patient (M.R.), rheumatoid-factor activity was demonstrated in γM containing immunoglobulin deposits.

Elution studies

Acid buffer eluates were prepared from biopsy and two kidneys from necropsy which showed no histological or clinical evidence of renal disease. Immunofluorescent study of these tissues revealed a linear de-

posit of γG-globulin. The eluates obtained did not contain demonstrable anti-nuclear or anti-basement membrane antibodies.

Discussion

Immunofluorescent study of renal biopsies and kidneys obtained at necropsy indicates that most patients with SLE have renal deposits of immunoglobulins and complement located within the mesangial areas of the glomerulus. The mesangium, which has been shown experimentally to contain cellular elements capable of phagocytic activity,[7] represents a primary filtering mechanism of the kidney. This structure may be the initial target for certain types of circulating immune complexes.[8] A mesangial deposition of protein also has been described in the early stages of NZB disease of mice,[9] which manifest a form of renal disease [10] that bears striking analogies to the nephritis of SLE found in man.

GBM injury, as indicated by the presence of proteinuria, is associated with granular deposits of immunoglobulins and complement in the early stages of the disease and with lumpy deposits of these proteins in the more advanced cases of glomerulonephritis. The factors which direct the basement membrane localization of complexes are unknown, although several hypotheses should be considered. The mesangium may be saturated with material which then lodges at the GBM. A qualitatively different type of immune complex with strong affinity for the GBM may be formed at certain stages of the disease and localize primarily on the GBM without entering the mesangium. Differences in the size of the complexes,[8,11] the ratio of antigen to antibody in the complex,[12] and the nature of the antigen-antibody system [13] involved may influence the site of glomerular localization of complexes.

Linear localization of γG-globulin in glomeruli is analogous to the pattern of staining observed for anti-basement membrane antibodies in Goodpasture's syndrome.[14] The linear deposits in SLE, however, are not associated with the concurrent localization of complement, nor are there microscopic or clinical findings of glomerular injury. Although there is no evidence that the linear deposits are cytotoxic, they may sensitize the basement membrane for the localization of anti-γglobulin antibodies, or immune complexes. Kidneys obtained at necropsy from patients with SLE glomerulonephritis have not demonstrated linear staining nor have anti-basement membrane antibodies been eluted.[6]

Attempts to elute the γ-globulin from glomeruli with linear staining and characterize its specificity were unsuccessful. It is possible that

antigen removed during the elution procedure, collagen, for example, blocked reactivity of the eluate. Localization of antibodies to collagen along the GBM may be responsible for the linear staining pattern. These antibodies have been demonstrated experimentally to localize in the kidney without cytotoxic effect,[15] and cytotoxic anti-GBM antibodies have been shown to be directed against antigenic determinants other than collagen in the basement membrane.[16] Linear deposits of γ-globulin also have been found in the basement membrane of normal skin obtained from patients with SLE.[17] Deposits of γG-globulin, but not complement, were found in the cutaneous basement membrane of 2 patients with linear deposits of γG-globulin in glomeruli. These findings raise the possibility that circulating non-cytotoxic antibodies to a variety of basement membrane antigens are formed in these patients.

Gamma-G-globulin was the predominant immunoglobulin noted in glomeruli of renal tissue obtained either at biopsy or necropsy. In one renal biopsy, an excess of γM-globulin was observed and, in this instance, material with rheumatoid-factor activity was detected. This may indicate that γ-globulin anti-γ-globulin complexes were present in glomeruli. Circulating 7S–19S cryoglobulins with rheumatoid-factor activity have been well documented in patients with SLE [18] and may exert cytotoxic effects. Vasculitis and focal glomerulonephritis have been associated with circulating 7S–19S cryoglobulins in the absence of SLE.[19] Rheumatoid factor may also fix to preformed complexes bound in the kidney and enhance the glomerular injury in a manner similar to the exacerbation of disease which follows the administration of rheumatoid factor to animals with Masugi nephritis.[20]

This study suggests that immunofluorescence examination of the renal biopsy may be a clinically useful method for assessing the extent of renal disease. The development of renal disease in several patients 3–8 years after the onset of SLE indicates that glomerular injury may not be an initial disease manifestation. It is therefore important to evaluate the nature of the renal immunoglobulin and complement deposits in "nonrenal" SLE. Whereas the presence of mesangial deposits are frequent, the appearance of granular deposits should be considered evidence of significant renal injury. Patients with granular deposits in glomeruli are presently under observation to determine if these lesions will resolve, when adequate immunosuppressive steroid or antimetabolite therapy is maintained. At present the prognosis for patients with mild glomerular lesions is not firmly established. Experimental evidence indicates that immune-complex type glomerular deposits are

at least partially reversible following cessation of immunization with foreign serum proteins.[12]

Summary and Conclusions

Renal biopsies from 19 patients were examined for the presence of immunoglobulins and complement. Marked differences in staining patterns were encountered. Linear deposits of γG-globulin without concurrent localization of complement were observed in three kidneys; solitary mesangial deposits of immunoglobulins and complement were found in five. The linear and mesangial deposits were not associated with clinical or histological evidence of renal disease. Granular and lumpy deposits of immunoglobulins and complement were associated with proteinuria and histological evidence of glomerular injury.

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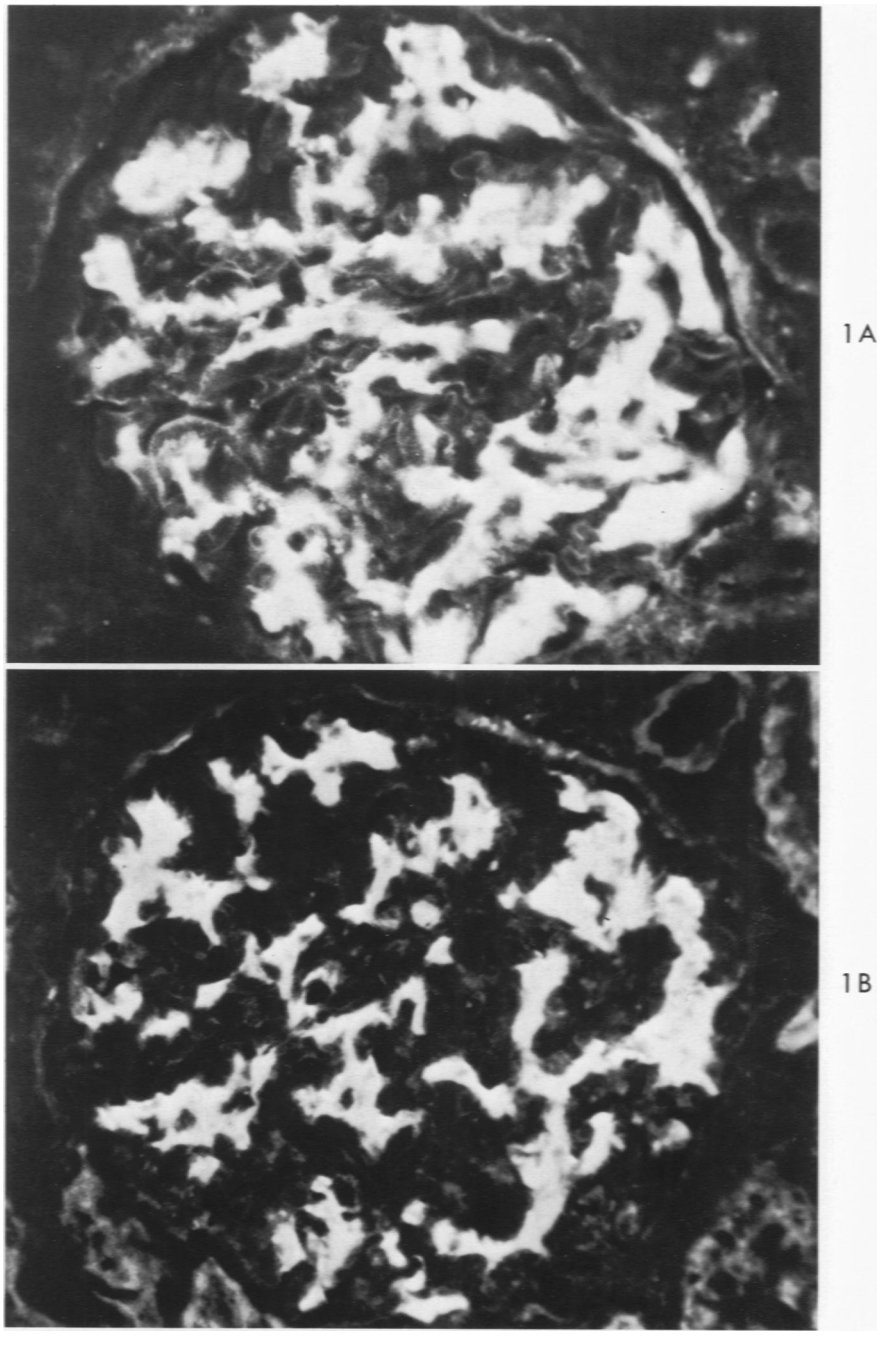

Fig 1. Patient P.W. (No proteinuria). Mesangial deposits of protein shown as irregular strand-like areas of fluorescence lying between capillary loops. **A.** γG-globulin localization. **B.** β_1C-globulin localization. × 250.

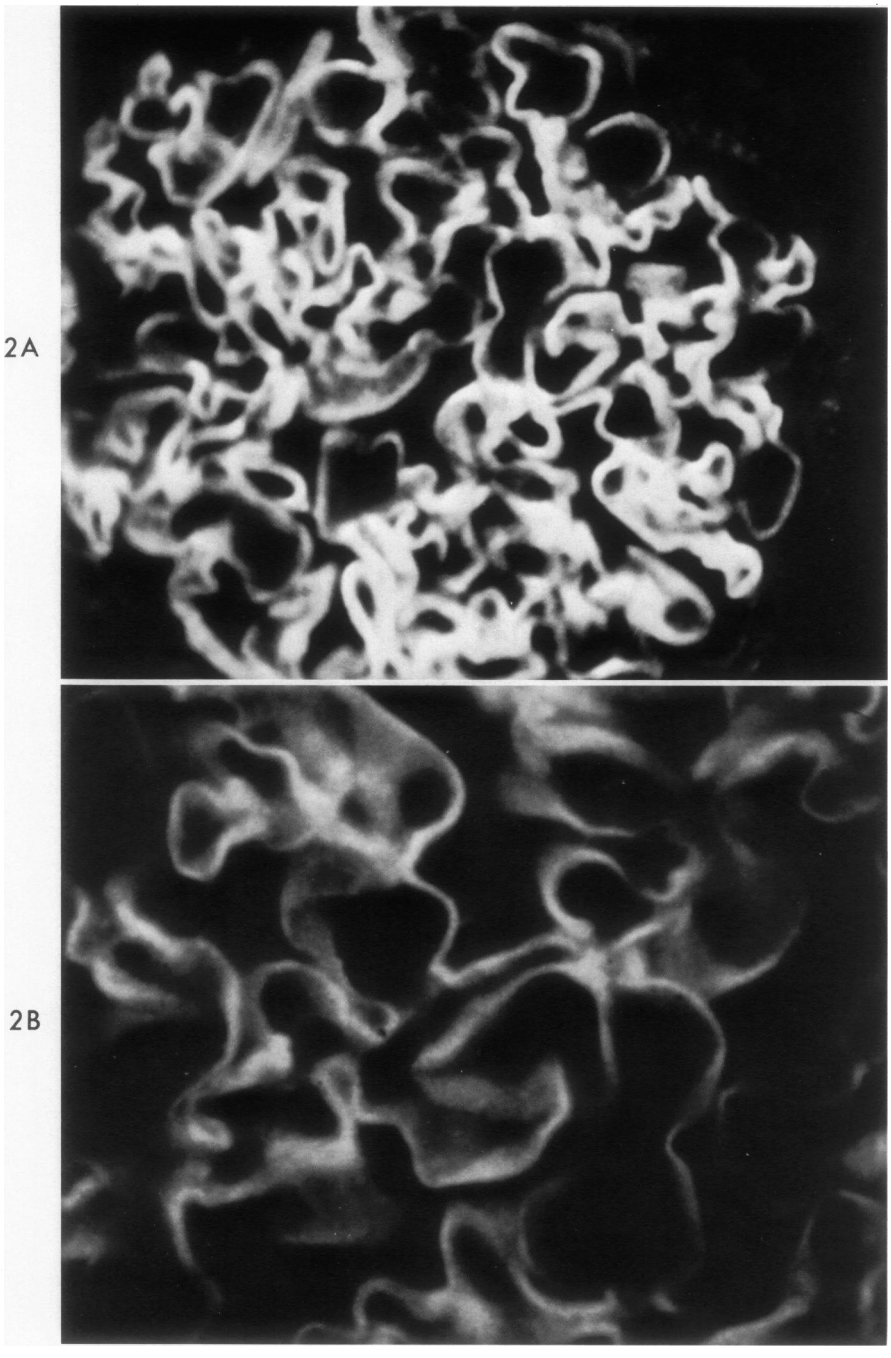

Fig 2. Patient D.S. (No proteinuria). Linear deposits of γG-globulin along GBM. Mesangial deposits of protein were not prominent in glomeruli of this biopsy. **A.** Pattern of γG-globulin deposition. × 250. **B.** Higher power of same glomerulus demonstrating homogeneous linear pattern of GBM staining. × 540.

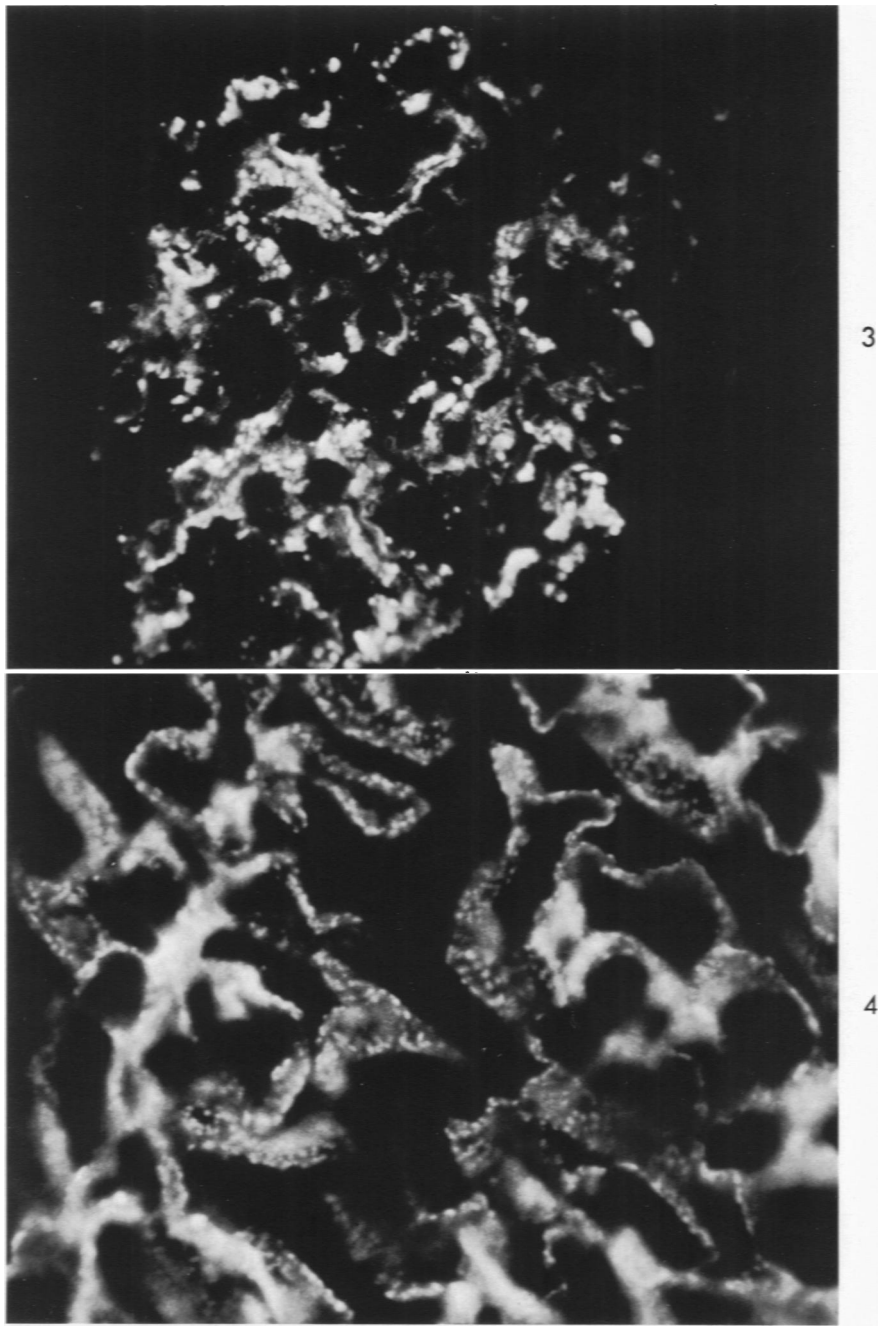

Fig 3. Patient J.B. (Moderate proteinuria with normal BUN). Characteristic granular deposition of γG-globulin along GBM. Note granules are of varying size and are distributed irregularly throughout glomerulus. × 250.

Fig 4. Patient H.W. (Marked proteinuria with nephrotic syndrome). High power view of a segment of glomerulus showing regular granular deposition of γG-globulin distributed along GBM. Contrast distribution with that noted in Fig 3. Note additional mesangial deposits of protein. × 540.

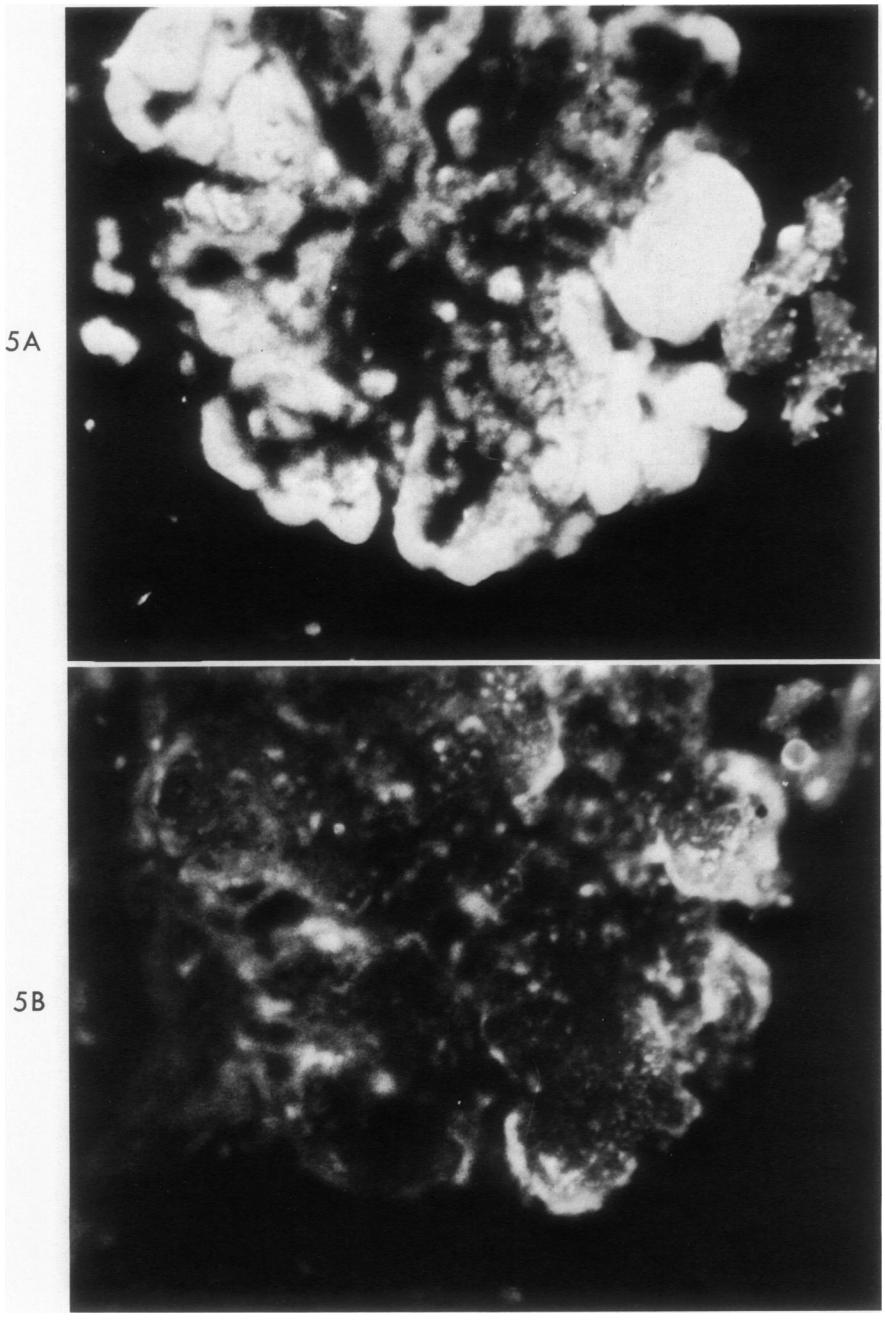

Fig 5. Patient M.R. (Marked proteinuria with increased BUN). Lumpy deposits of immunoglobulin outlining glomerular tufts. No granular deposits are discernible. **A.** Marked deposition of γM-globulin. **B.** Minimal deposition of γG-globulin shown in serial section of same glomerulus. × 250.